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**TECHNICAL MEMORANDUM**

TO: Distribution

FROM: Kirby Tyndall, Ph.D., DABT
Senior Consulting Toxicologist

DATE: December 3, 2010

RE: Meeting Notes from December 1, 2010 Ecorisk Meeting
Gulfco Marine Maintenance Superfund Site

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Baseline Ecological Risk Assessment (Preliminary Site Characterization Report) meeting with Larry Champagne, Luda Voskov, Susan Roddy, Eric Pastor, Steve Brown, Margaret Roy, Kirby Tyndall, Michael Jones, Jim Horne, and Ron Gouguet

Eric reviewed objectives as identified in the meeting agenda (see Attachment A). The overall goal of the meeting was to work through the data presented in the PSCR (e.g., toxicity data) so that the team can understand how the data relates to finalizing data questions/uncertainty in the ecological risk process. The ultimate goal was for the BERA to meet the expectations of the team and minimize re-evaluation and re-interpretation of the data in the BERA.

Jim Horne discussed toxicity testing. Test program consisted of whole sediment toxicity tests with two invertebrates, soil toxicity testing with one invertebrate, and toxicity testing of surface water for an aquatic invertebrate, as proposed this summer. Deviation from toxicity testing protocols for soil and surface water was necessary because of the high salinity, which was not suitable to the test species. Each test group included a lab control (negative control) to show that the organisms were fit for use and that the test system was "in control". Material (sediment) for the lab control originates from the York River, Virginia (near Chesapeake Cultures, Inc., the source of the test organisms). The sediment is processed by sieving to 0.5 mm to remove debris and indigenous macroinvertebrates, and is frozen for preservation. This material was used for the control medium in the polychaete and amphipod tests (this material is commercially sold for this purpose). Susan asked what is the growth threshold used for the acceptance criteria. Jim did not recall at the time but stated that the TAC details were contained in report (Jim checked after the meeting, and the *Leptocheirus* method requires a minimum mean control survival of 80% and measurable growth and reproduction in all control replicates). Jim indicated that the data showed that the sediment control organisms were appropriate and met the acceptance criteria.

Jim described the CETIS statistical package and decision tree…the decision tree comes from the 2005 EPA sediment assessment manual (and sometimes an additional decision tree such as from



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the *Leptocheirus* guidance document, when other alternatives are available). The other evaluation conducted with site sediment is compared against the reference sample. Biomass and dry weight were two measurements available to describe growth. Larry was wondering if we used both measurements equally to describe growth effects. Margaret indicated that only dry weight was considered, and offered an explanation of why this is appropriate. Larry saw some vastly different outcomes for biomass vs. dry weight vs. control. Susan asked if there is a way to sort out data by similar organic carbon and grain size. Jim responded that there are numerous ways of doing this to normalize outcome but we need to come to an agreement on how we do that. Larry asked why this was not indicated in the PSCR if the biomass part of growth endpoint was different. Margaret responded that the footnote in the Tables follows EPA guidance and provides information on why biomass is less important than the dry weight and, therefore, that's what was reported. Both are reported in summary tables, but conclusions are based on dry weight (due to the referenced guidance). Susan noted that mortality is captured in the biomass measurement.

There were some concerns about the statistical package used and why there is toxicity in reference samples. When the statistics seem questionable, it usually can be explained by realizing that the values in the tables are means, and that the actual data tests each contained five replicates with 5 or 20 organisms in each replicate (depending on the test). As such, some of the data distributions can overlap and appear statistically similar even when their means are not similar. Jim explained this concept with the statistical graphs in appendices and other summary stats.

Steve explained why you compare site samples vs. reference samples and not vs. lab controls, emphasizing that the only purpose of the laboratory control data is to verify that the test organisms were healthy and the tests were conducted properly. Steve said that, in fact, the laboratory control data should really only be considered once to assess, determine and verify the validity of the toxicity tests, and then all subsequent comparisons (i.e., in data tables) should be for Site data vs. reference. He cited EPA's 2005 Contaminated Sediment Remediation Guidance at Hazardous Waste Sites. We looked at the lab output in the appendices to help understand the variability and the data. Susan is concerned with the high zinc concentration in soil south of the Fresh Water Pond, and she was concerned with why a reference location could/would have 19% or 33% survival. Steve indicated that it's very likely that the physical nature of the sediments at the site and reference locations precludes it from being a good medium for the survival and growth of these organisms (ie., too course-grained, too little TOC, too much clay, etc.), but it is probably not likely a result of the site-related compounds since COPEC concentrations in the reference locations were low. Larry agreed with the TOC issue but takes issue with the grain size. Steve stated that he has learned through experience that benthic organisms, including *Leptocheirus*, can be fairly sensitive to grain-size distribution and TOC.

Susan suggested that looking at the data normalized over the TOC and grain-size distribution would help with the BERA rationale. Steve indicated that we certainly could, but we should frame the question is there any site-related compound that would be driving the observations we are seeing at the site. Total PAH would not support this; even high zinc concentrations generally do not support it either.

Larry indicated that he originally thought that the pore water data might suggest a "problem", but that falls apart when you look at ICWW pore water and toxicity data and compare that with the same results for the wetlands sediment toxicity and pore water data....there was not comparable pore water issues between wetlands and ICWW but similar toxicities (poor animal performance at both locations). Larry has never seen variability in data like this. Steve agreed...if we handpicked sediment, we might be able to constrain the variability. Susan indicated that the

reference locations analytically indicate they are good reference locations. Physically, there is good agreement between the sediment collected at the reference locations and the site locations.

Jim also mentioned that this was a 28-day test which is a whole different beast than shorter tests, and sometimes provides different results. Larry asked if they process the site sediments to make it more hospitable for the test organism. Jim indicated that, no, typically the sediments are minimally processed, and mostly to remove detritus. These samples were press-sieved to get rid of predator invertebrates (only true for ICWW sediment). Soils that were treated as sediment had a lot of clay and therefore had to go through a grater so that once it was hydrated, it would at least resemble a sediment. But that was the extent of the processing for site and reference sediments; both were "processed" in the same manner when it was necessary.

Eric reminded the group that the wetland sediments are sometimes under water but sometimes they are completely dry as well. It was noted that during sampling, sometimes the samples would be dry from 6 inches to a foot, and for many of the wetland sediments, it was extremely difficult to obtain pore water, except for after a rain event. It was discussed that these conditions can be physically stressful for an organism.

Larry indicated that COPEC concentrations were of concern for some compounds at some locations when looking at analytical data (total concentrations in whole sediment) compared to screening criteria. Steve agreed, but noted that constituent the observed low concentrations in pore water indicate that the COPECs are not bioavailable or toxic. Michael pointed out that, while there might be locations where there were relatively high concentrations of some constituents, the toxicity data do not suggest there are areas of unacceptable risk.

**North Area soils**. There is some overlap between soils and sediments. Toxicity testing resulted in acute toxicity in earthworms most likely due to high salinity in soil. Therefore, a modified *Neanthes* test for 21-days was used to assess toxicity from soil exposure. Three approved reference locations (from same area as original background soil sampling was conducted) were tested. Poorest survival was in reference locations (92% survival for sample NAS07 where zinc was detected at 501 mg/kg; 64% survival for sample NAS08 where zinc was detected at 182 mg/kg; and 60% survival for sample NAS09 where zinc was detected at 63.1 mg/kg) ; site survival ranged from 76% to 96%. Somewhat mirrored the growth. P-value is listed as well. Significant is less than 0.05; not significant if the P-value is greater than 0.05.

Conclusion for the soil is that there is no adverse effect seen when comparing site samples to reference samples. There was agreement by the group with this conclusion.

**ICWW sediment.** Fairly low concentrations of COPECs in Intracoastal Waterway sediment; reference locations had even fewer detections. Very few constituents were detected in the pore water for either site or reference sediment. It was generally noticed that the concentrations in the BERA data set were less than the original RI data. *Neanthes* test results indicated good survival (Table 3 of Final PSCR is very useful). *Leptocheirus* survival was not as great, but Jim explained that they start out younger, smaller (2 mm in length) and aren't as hardy (*Neanthes* are a similar age but much bigger, 4-6 mm in length and more highly developed). Larry pointed out that lab control differences highlight the differences in hardiness between the two test organisms. Consistent survival across the site and reference samples suggests no site-related compounds are driving toxicity; there was agreement by the group with this conclusion.

**Wetlands sediment.** There were some localized higher concentrations of a variety of COPECs, but again these concentrations were consistently a little lower than RI data set. Figure 4 and

Table 3 provide a summary of the data. *Neanthes* survival was better than *Leptocheirus* survival. Reference location survival for *Leptocheirus* was fairly low. A correlation between concentration gradient vs. toxicity was not apparent. The highest concentration sample for PAHs was closest to the road where no pore water could be collected. In general, sediment concentrations of total PAHs were found to be low compared to sediment quality values (i.e., < 10 mg/kg vs. ER-M of 44 mg/kg). A few pore water measurements at a few stations were found to exceed the continuous chronic criterion values for certain metals (copper at EWSED03, EWSED04 and EWSED06, and zinc at EWSED04 and EWSED06). Also, EWSED08 (reference location) had a pore water exceedance for nickel and (perhaps) endrin aldehyde (J flag). EWSED07 and EWSED09 did not have pore water exceedances.

So, Steve emphasized that pore water concentrations indicate that potential exposure to bioavailable constituents is very limited, and these data do not correlate well to the observed sediment toxicity data, suggesting that factors other than chemical constituents (i.e., sediment type, low TOC) were likely responsible for the poor performance that was observed in Leptocheirus tests. Therefore, it was concluded and agreed that ecological risks associated with Site sediments, based on the bioavailable fraction, is low.

It was suggested that it would be worth trying a multivariate analysis of the different chemical and physical parameters to help elucidate the drivers for adverse effects and that the analysis should be run for reference and site samples. The group was in agreement that this will be done for the BERA and that the data suggest no site-related compounds are driving toxicity although it is difficult to discern what is causing toxicity. It was discussed that observed adverse effects may be due to different factors or a combination of factors, and may include physical parameters such as sediment type, bioavailability, and low TOC and/or the chemical concentrations present in the sediment.

**Wetlands surface water.** Salinity in surface water would be detrimental to the proposed mysid shrimp survival so brine shrimp (*Artemia spp.*) were used, with a modified and longer than typical protocol as previously discussed. Three samples collected. The reference location was too dry and, therefore, no sample was collected. Acrolein was not detected; copper was detected in SW01. Jim indicated that, unfortunately, brine shrimp are not very sensitive and do not withstand much after 48 hours. Therefore, it is hard to get reliable performance with them in toxicity tests. Susan asked if that is why there were three test runs. Jim answered yes and indicated that the test was not worth running at 96 hours. It was noted that control survival at 48 hours met the acceptance criteria for the test. Steve noted that the data show that we have copper in pore water exceeding the chronic continuous criteria by factor of 2 at one station (EWSW03); barely exceeds at another location (EWSW01); and not at the other. Ron said that we probably should be comparing to acute criteria since the test was an acute duration and given the fluctuating conditions of surface water at the site. The group agreed. It is expected that there are no surface water quality exceedances when comparing to acute AWQC. Jim noted that at 48 hours, the survival rate of the brine shrimp was good. Therefore, surface water most likely does not suggest adverse site-related effects. Larry suggested that, in the BERA, this issue be summarized comparing data to acute criteria and presenting the data at 48 hours. The LC50 analysis described in the PSCR will not be presented in the BERA since it serves no purpose.

Conclusions for the surface water are that there is limited usefulness in the 96-hour toxicity testing that was conducted because of control survivability issues, and that the survival rate at 48-hours suggests that there are no site-related effects. The detected concentrations will be compared to acute criteria, the LC50s will be removed from the BERA, and the results from the 48-hour testing will be discussed. There was agreement by the group regarding these items.

**Bigger Picture Discussion.** For the BERA, a multivariate analysis of the data will be presented for the wetland sediment in an effort to try to assess any potential relationships between sediment characteristics (e.g., grain size distribution, TOC, constituent concentrations) and toxicity test organism performance. Because of the small dataset, the multivariate analysis may not prove to be particularly powerful, but it should be developed and presented in the BERA.

Steve noted that it seemed that the biggest stumbling point in the beginning of the meeting was the apparent disconnect between poor survivorship and growth for *Leptocheirus* exposed to sediments from the Site vs. laboratory controls, which we now agree is an improper comparison. The laboratory control data is used only for the purpose of validating that the test was run properly. The proper comparison is between results for the Site samples vs. the reference samples, and there were not significant differences in test organism performance between the Site stations and reference stations, acknowledging that data variability was an important factor. Steve asked if there is still an issue with working through this problem. Susan said she has a clearer understanding of the data. Larry asked whether the use of a reference sample is an optional part of sediment toxicity testing? Michael and Steve and Jim all noted that both EPA and ASTM guidance documents specifically call for the use of reference samples and define what a reference site is. Steve summarized that based on the data and our discussion of the data, the conclusion is that there are no significant relationships between site-related constituents and organism responses observed in the toxicity tests. There was general agreement on this point.

Larry is comfortable with reference compared to site samples. He would like the BERA to provide a defensible rationale on why the reference samples are appropriate (i.e., analytical data suggest that these are not impacted by the site, etc.).

Ron pointed out that it sounds like we are in agreement that there are no big issues to be addressed and that NOAELs/LOAEL-based PRGs will not be developed or presented in the BERA for any of the areas. All were in agreement with this statement.

Jim indicated that if exposure to site COPECs were the source of poor survival, the variability in the toxicity data would be lower and the adverse effects would be much more apparent because the specimens would have died or failed to thrive; in fact, poor performance in the tests should have been correlated with variable COPEC concentrations in the samples. Since that was not the case, it follows that chemical stressors were not present in the site samples at sufficient levels to explain the poor performance and high variability; thus, the observed poor performance must have been due to other factors. Larry noted, however, that if a physical stressor or other factor causes an adverse effect sooner, it is impossible to measure or observe the effect a constituent(s) may or may not have on the test. It was also discussed that the results of the toxicity testing contradict one of the assumptions of the BERA Work Plan (i.e., differences in the toxicity test results between the reference/background samples and Site samples are a result of differences in concentrations or bioavailabilty of the COPECs in the media). Susan said she would like to see this information in the BERA.

Larry wrapped up his comments by saying that the comparison between site samples and reference samples trumped any of the issues he may have had about the data set. He suggested that the BERA included playing up the dataset variability and would prefer to not see lots of reference to toxicity. There was a brief discussion after Larry wondered what it would have looked like if we would have used a shorter time duration for these tests. The group agreed to remove the summary text in the tables about the comparison of the laboratory control to the reference samples.

Susan would like to see some of these lessons learned from this Site in the Uncertainty Section. Ron stated that he thinks this would be handled better in a different forum.

It was concluded that the meeting provided useful dialogue and helped everyone better understand the data set, and that the data suggest that toxicity observed in site and reference samples are not due to site-related COPECs in any media.

**ATTACHMENT A – December 1, 2010 Meeting Agenda**

**Gulfco Marine Maintenance**
**Superfund Site**
**Freeport, Texas**
**Preliminary Site Characterization Report (PSCR) – Meeting Agenda**

**Objective** – Review the data generated from the Baseline Ecological Risk Assessment (BERA) field work, as presented in the PSCR. This meeting provides an open forum for a detailed review and discussion of the data and its meaning prior to the development of the BERA Report.

1.0 Project Review – Work plan development/implementation and its consistence with EPA Ecological Risk Assessment process, guidance and approvals.

2.0 Presentation of Data by Study Area
- 2.1 North Area Soil
  - 2.1.1 Analytical Chemistry Results
  - 2.1.2 Toxicity Testing Results
  - 2.1.3 Conclusions
- 2.2 Wetland Sediment
  - 2.2.1 Analytical Chemistry Results
  - 2.2.2 Toxicity Testing Results
  - 2.2.3 Conclusions
- 2.3 Surface Water
  - 2.3.1 Analytical Chemistry Results
  - 2.3.2 Toxicity Testing Results
  - 2.3.3 Conclusions
- 2.4 Intracoastal Waterway Sediment
  - 2.4.1 Analytical Chemistry Results
  - 2.4.2 Toxicity Testing Results
  - 2.4.3 Conclusions

3.0 Discussion of Development of the BERA Report